

3. (Amended) A method to produce hybrid seed with restored male fertility [from plants selected from those species of pollen producing plants which are capable of being genetically transformed] comprising the steps of:

- (a) inserting into the genome of a plant cell of said pollen producing plant a gene which confers on said plant resistance to a chemical agent or physiological stress, and linked to said gene a recombinant DNA molecule comprising:
 - (i) a DNA sequence which codes for a cytotoxic molecule;
 - (ii) a [pollen targeted promoter which functions in said plant cell to cause] promoter capable of regulating the transcription of said DNA sequence in cells critical to pollen formation or function; and
 - (iii) a terminator sequence which defines a termination signal during transcription of such DNA sequence;
- (b) obtaining a transformed plant cell;
- (c) regenerating from said plant cell a genetically transformed plant which is male sterile;
- (d) increasing the number of genetically transformed plants by:
 - (i) crossing the genetically transformed plant described in step (c) above with a suitable male fertile plant;
 - (ii) using a chemical agent or physiological stress to eliminate plants which do not contain the genes described in step (a) above among plants grown from seed produced by such cross; and
 - (iii) repeating such a cross over several generations with the plants obtained as in step (d)(ii) above in the presence of said chemical agent or physiological stress to increase the numbers of male sterile plants;

- (e) inserting into a plant cell of a suitable male fertile plant selected from the same species a gene which confers on said plant resistance to a chemical agent or physiological stress and linked to said gene a recombinant DNA molecule comprising:
 - (i) a DNA sequence which codes for RNA that is complementary to the RNA sequence coding for said cytotoxic molecule;
 - (ii) a promoter which causes transcription of the DNA sequence defined in step (e)(i) above at or about the time of transcription of the DNA sequence defined in step (a)(i);
 - (iii) a terminator sequence which defines a termination signal during transcription of the DNA sequence described in step (e)(i) above;
- (f) obtaining a transformed plant cell from step (d);
- (g) regenerating from said transformed plant cell described in step (d) above a genetically transformed male fertile plant; and
- (h) producing a restorer line by:
 - (i) selfing the genetically transformed plant described in (g) and selecting from that selfing progeny, a plant homozygous for the male restorer trait;
 - (ii) permitting self-fertilization of said plant homozygous for the male restorer trait; [and]
 - (iii) growing seed of said plant, over a number of generations to increase the number of genetically transformed plants; and
 - (iv) effecting a hybrid cross by pollinating said male sterile plants with pollen from said genetically transformed male fertile plants.

4. (Amended) A method of producing hybrid seed with restored male fertility [from those species of pollen producing plants capable of being transformed and regenerated into a differentiated whole plant which method comprises] comprising the steps of:

- (a)
 - (i) inserting into the genome of a plant cell of said plant that is capable of regeneration into a differentiated whole plant, a sense gene and linked to this a recombinant DNA molecule comprising:
 - A. a DNA sequence that when transcribed and translated codes for a cytotoxic molecule [of] or a molecule which breaks down a substance into a cytotoxic molecule;
 - B. a [pollen targeted promoter which functions in said plant to cause] promoter capable of regulating the transcription of said DNA sequence into RNA at or about the time of the transcription of the sense gene in [developing pollen] cells or tissues critical to pollen formation or function; and
 - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;
 - (ii) obtaining a transformed plant cell of said plant; and
 - (iii) regenerating from said plant cell a plant which is genetically transformed with said DNA sequences described in (a)(i) above and is male sterile; and
- (b) increasing the number of genetically transformed male sterile plants by:
 - (i) clonal propagation of said genetically transformed male sterile plant described in step (a) using tissue explants thereof, or other [in vitro] in vitro propagation techniques; or
 - (ii) A. crossing the genetically transformed male sterile plant described in (a) with a isogenic male fertile plant;

- B. using [the] a chemical agent or physiological stress [defined in (a)(ii) above] to eliminate plants which do not contain the DNA sequence defined in (a)[(ii)] (i) amongst plants grown from seed produced by such cross; and
- C. repeating such cross over several generations with plants obtained in step [(b)(iii)] (a)(iii) above in the presence of said chemical agent or physiological stress to increase the numbers of male sterile plants;

(c) producing a male fertile restorer plant by:

- (i) inserting into the genome of a plant cell of a suitable male parent plant that is capable of regeneration into a differentiated whole plant a gene that confers resistance to a chemical agent or a naturally occurring or artificially induced physiological stress, linked to a recombinant DNA sequence comprising:
 - A. a gene that codes for a molecule that negates the disruption caused to cells or tissues critical to pollen formation or function in said genetically transformed female parent plant;
 - B. a promoter that functions in said cells or tissues critical to pollen formation or function to cause transcription of said gene into RNA at or about the time that the sense gene described in (a)(i) is active; and
 - C. a terminator sequence which defines a termination signal during transcription of said DNA sequence;

- (d) increasing the number of genetically transformed male fertile restorer plants by:
 - (i) selfing the genetically transformed plant carrying the restorer trait described in (c), and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation; or
 - (ii) [when applicable,] conducting anther or isolated microspore culture of the genetically transformed plant carrying the restorer trait described in [C.] (c) and selecting a plant homozygous for the restorer trait and increasing said plant by selfing in isolation [.] ; and
- (e) optionally effecting a hybrid cross by pollinating said male sterile plants described in (a) and increased in (b) in the presence of the chemical agent or physiological stress [defined in (a)(ii), (if required),] with pollen from male fertile restorer plants as described in (c) and increased in (d).

5. (Amended) A method of producing hybrid seed [from a plant selected from those species of pollen producing plants which are capable of being genetically transformed, which method comprises] comprising the steps of:

- (a) inserting into a genome of one or more plant cells of said plant the recombinant DNA molecule comprising:
 - (1) one or more DNA sequences [which may be the same or different], which encode a gene product which when produced in a cell of a plant which is [essential] critical to pollen formation [and/]or function is [directly or indirectly] capable of substantially interfering with the function [and/]or development of said cell; and
 - (2) one or more promoters [which may be the same or different, said promoters being] capable of regulating the [expression]

transcription of said DNA sequences in cells critical to pollen formation or function;

[wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and] whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait; and

- (b) selecting a plant cell into which the recombinant DNA molecule is stably integrated;
- (c) regenerating from the selected plant cell a plant which carries the male sterile trait;
- (d) [where appropriate,] optionally increasing the number of plants which carry the male sterile trait by selfing a plant which carries the male sterile trait, selecting a plant homozygous for the male sterile trait and increasing the homozygous plant by selfing in isolation, or by conducting anther or isolated microspore culture of a plant which carries the male sterile trait to select a plant which is homozygous for the trait and increasing said homozygous plant by selfing in isolation;
- (e) exposing said plant or plants to a sterility actuating agent which renders said plant or plants male sterile; and
- (f) crossing said male sterile plant or plants so obtained with a male fertile plant without said recombinant DNA molecule to obtain hybrid seed, said male fertile plant is selected such that the hybrid seed is capable of growing into a male fertile plant, said male fertile plant has a restorer DNA molecule encoding a restorer gene product integrated into its genome, said restorer DNA molecule or said restorer gene product being capable of restoring the function [and/]or development of [a cell/tissue] cells of a plant [that is]

essential to pollen formation [and/or function that [is] are selectively interfered with by said gene product encoded by said DNA sequence of said recombinant DNA molecule.

6. (Amended) A method of producing hybrid seed [from a plant selected from those species of pollen producing plants which are capable of being genetically transformed which method comprises] comprising the steps of:

(a) inserting into the genome of one or more plant cells of said plant a recombinant DNA molecule comprising:

(1) one or more DNA sequences [which may be the same or different,] which encode a gene product which when produced in a cell of a plant which is [essential] critical to pollen formation [and/or function is [directly or indirectly] capable of substantially interfering with the function [and/or development of said cell; and

(2) one or more promoters [which may be the same or different, said promoters being] capable of regulating the expression of said DNA sequences in cells critical to pollen formation or function;

[wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and] whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially male-sterile or carries the male sterile trait; and

(b) selecting a plant cell into which the recombinant DNA molecule is stably integrated;

(c) regenerating from the selected plant cell a plant which is male sterile;

(d) increasing the number of male sterile plants to produce a male sterile line by clonal propagation or by crossing the male sterile plant with a suitable male fertile plant and selecting plants which

express said recombinant DNA molecules and repeating the steps of crossing and selecting as required using progeny of such cross(es) which have been selected; and

(e) crossing said male sterile line with a suitable male fertile plant line without said recombinant DNA molecule to obtain hybrid seed, said male fertile plant line is selected such that the hybrid seed is capable of growing into a male fertile plant, most plants of said male fertile plant line have at least one restorer DNA molecule encoding a restorer gene product integrated into their genome, said restorer DNA molecule or said restorer gene product being capable of restoring the function [and/]or development of [a cell/tissue] cells of a plant [that is essential] critical to pollen formation [and/]or function that [is] are selectively interfered with by said gene recombinant DNA molecule.

7. (Amended) Hybrid seed of a pollen producing plant having a nuclear genome incorporating one or more recombinant DNA molecules comprising:

(a) one or more DNA sequences [which may be the same or different,] which encode a gene product which when produced in a cell of a plant which is [essential] critical to pollen formation [and/]or function is [directly or indirectly] capable of substantially interfering with the function [and/]or development of said cell; and

(b) one or more promoters [which may be the same or different, said promoters being] capable of regulating the expression of said DNA sequences in cells critical to pollen formation or function; and

[wherein the DNA sequences and promoters are selected such that the gene product selectively interferes with the function and/or development of a cell of a plant that is essential to pollen formation and/or function and] whereby a plant regenerated from a cell of a plant having said recombinant DNA molecule integrated into its nuclear genome is substantially